Supplemental Figure S1. $TNF^{\Delta ARE/+}$ mice display gender differences in disease severity and phenotype of GALT Tregs. (A) Histologic evaluation of ileal inflammation in young (<10 wks) and old (>10 wks) cohorts of $TNF^{\Delta ARE/+}$-M and –F mice (n=24/group) shows increased disease severity in females (left). Representative photomicrographs of H&E-stained ilea from 16-wk-old $TNF^{\Delta ARE/+}$-M and -F mice are shown (right; scale bar = 100 μm; X20 + 1.25 original magnification). (B) 16-wk-old $TNF^{\Delta ARE/+}$-M mice express an increased percentage of Treg cells ($CD25^+Foxp3^+$) within the MLN $CD4^+$ T cell population by flow cytometry (left, n=6/group). Representative histograms show CD25 and Foxp3 expression within the $CD4^+$ population (right).

Supplemental Figure S2. FACS sorting scheme for isolation of $CD45RB^{hi}$ and $CD45RB^{low}$ cells. $CD4^+CD45RB^{hi}$ and $CD4^+CD45RB^{low}$ cells were isolated from SAMP-M and –F mice by fluorescence activated cell sorting. Figure shows sequential gating on lymphocytes by FSC-A and SSC-A, followed by exclusion of aggregates by

SSC-W and SSC-A. Singlet lymphocytes were selected on CD4 positive fluorescence and then CD45RB high and low expression.

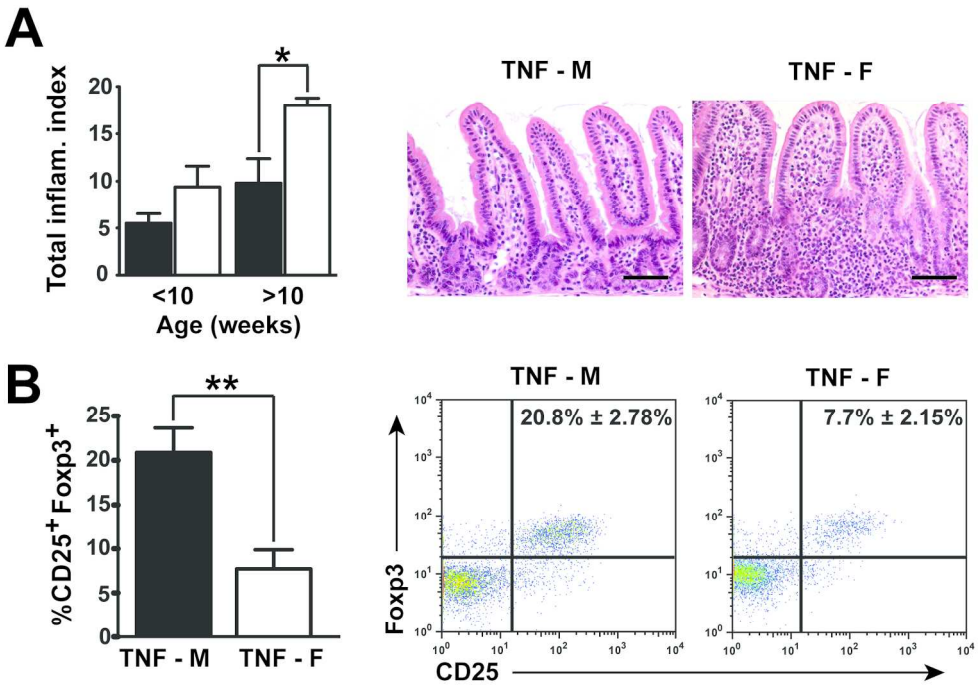
Supplemental Figure S3: Treg cells from SAMP-M show enhanced ability to suppress proinflammatory cytokines and MPO in *in vivo* adoptive transfer model. Colon tissues and MLNs were harvested from mice 8 weeks after adoptive transfer, and MLN cells were activated *in vitro* with αCD3/αCD28. MPO activity in colon tissues and cytokine secretion from activated MLN cells are expressed as mean ± SEM (*p<0.05 and **p<0.01 compared to $CD45RB^{hi}$ group; n=16/group).

Supplemental Figure S4. FACS sorting scheme for isolation of Tconv and Treg cells. $CD4^+CD25^-$ Tconv and $CD4^+CD25^+$ Treg cells were isolated from SAMP-M and –F mice by fluorescence activated cell sorting. Figure shows sequential gating on lymphocytes by forward (FSC-A) and side (SSC-A) scatter, followed by exclusion of aggregates by SSC-W (width) and SSC-A (area). Singlet lymphocytes were selected on CD4 positivity and then CD25 high expression (approximately top 10% of CD25-positive population).

Supplemental Figure S5. Serum estrogen levels are comparable in SAMP versus AKR mice, post-puberty. Serum estrogen levels were measured in SAMP and AKR

male and female mice at 6 and 14 weeks of age. Levels are presented as mean ± SEM; significance is based on two-tailed Student's t-test with Welch's correction (*p<0.05, n=8-10/group).

Supplemental Figure S6. Gender does not influence *in vivo* permeability. (A) *In vivo* permeability of 4-5 week old AKR and SAMP mice was assayed using the lactulose/mannitol (Lac/Man) ratio test. Data is expressed as the mean ± SEM (n=6-12 mice per group). (B) 12 week old SAMP-M mice were castrated and then injected i.p. for 6 weeks (daily, 200ul) with vehicle Ctrl (DMSO/PBS), E2 (10 μg/kg), PPT (10 mg/kg), or DPN (10 mg/kg). Lac/Man permeability tests were performed following 6-wk treatment and data is expressed as the mean ± SEM (**p<0.05, n=6 mice/group).



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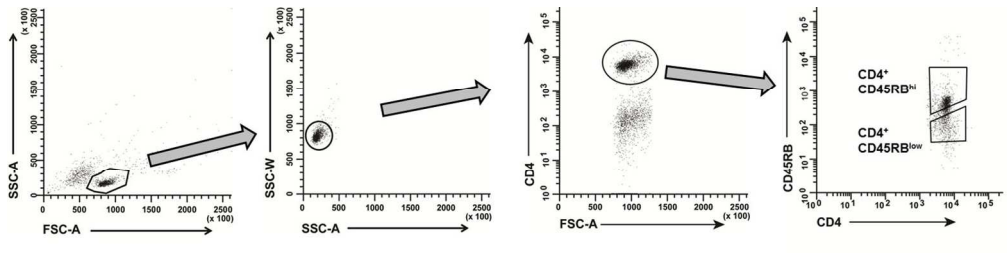

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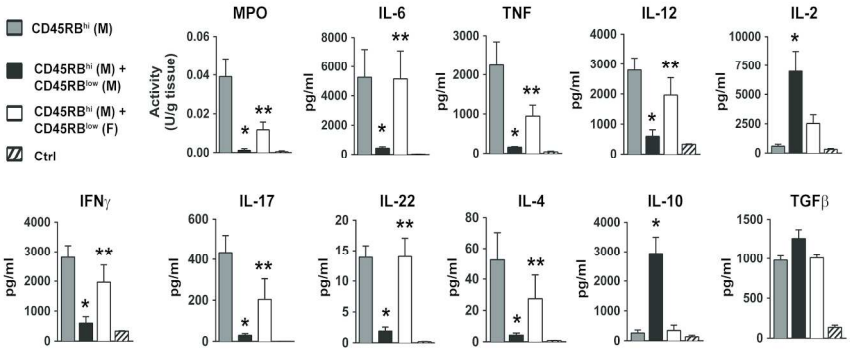

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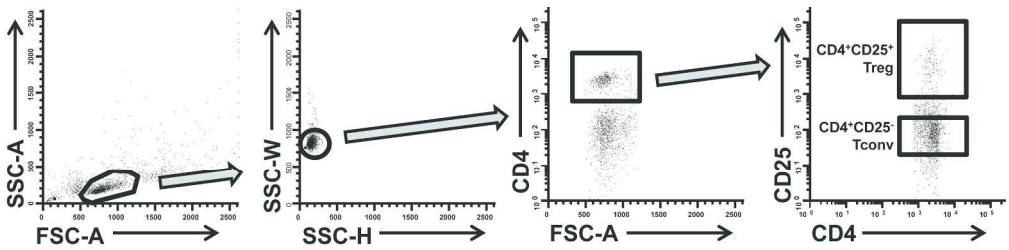

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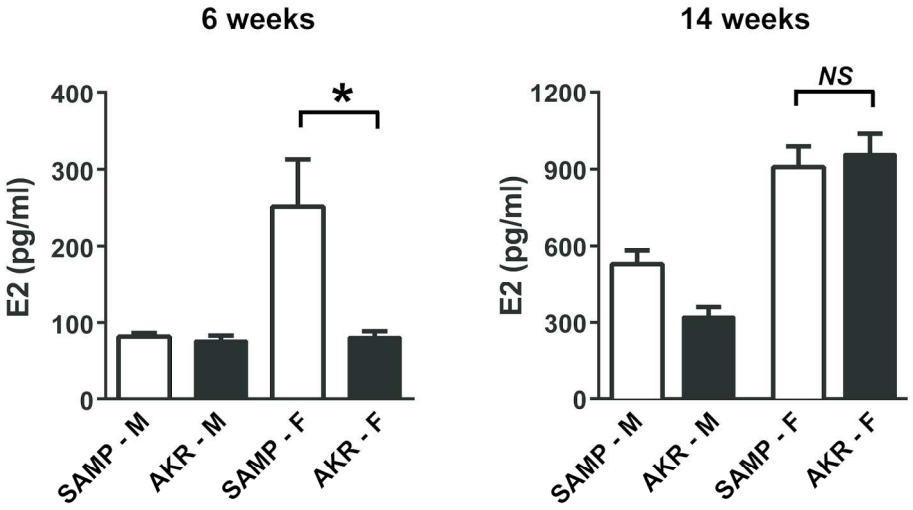

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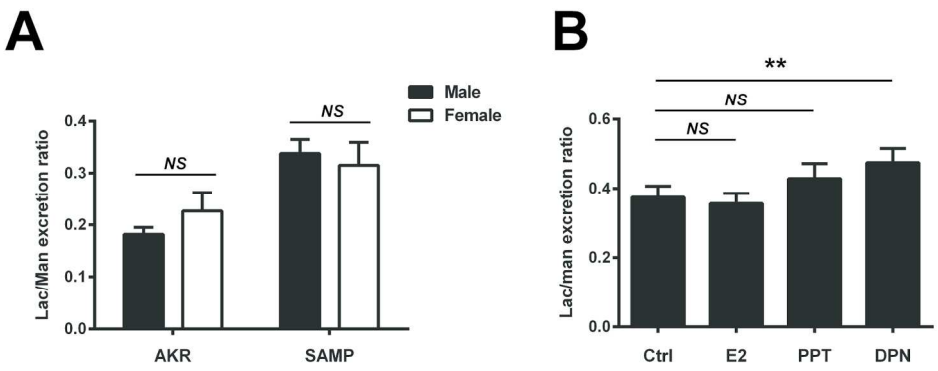

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